🧫 Identification of Bacteria

University of Kut

College of Dentistry

Practical Course in Medical Microbiology

Third stage lab 4

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Objectives of Studying Bacterial Characteristics

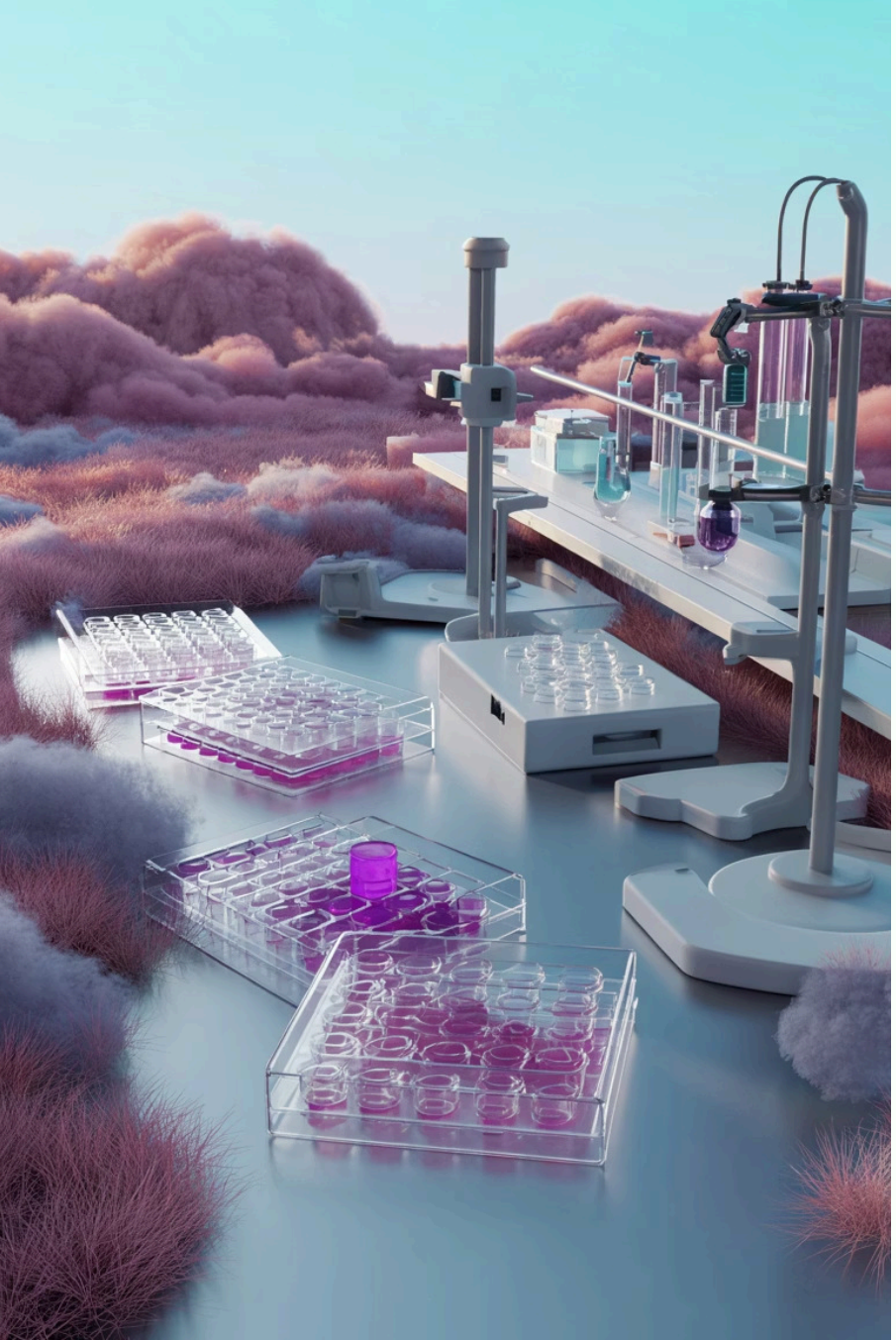
1- Identify and differentiate bacterial types

2- Diagnose microbial diseases

3- Select targeted treatments

4- Differentiate between beneficial and pathogenic oral bacteria.

5- Prevent and control infection



The Identification Process

Bacterial identification follows a systematic three-stage approach, each providing progressively more specific information about the organism.

Macroscopic / Cultural

Naked-eye observation of colonies on culture media

Microscopic

Morphology and staining characteristics under magnification

Biochemical

Metabolic pathways and enzyme production tests

Module 1: Macroscopic & Cultural Examination

Definition & Significance

Macroscopic examination is the initial step–studying bacterial colonies growing on culture media with the naked eye. This preliminary assessment reveals crucial information about bacterial identity, pathogenicity, and sample quality.

Identifies Bacterial Type

Colony appearance provides initial clues to genus and species identification.

Assesses Sample Purity

Distinguishes pure cultures from contaminated mixed cultures.

Predicts Pathogenicity

Certain colony characteristics correlate with virulence and disease potential.

Correlates with Clinical Origin

Colony type often reflects the oral source–plaque, gingival, or abscess flora.

Culture Methodology: Essential Steps

1

Prepare & Sterilize Media

Select appropriate culture medium and sterilize by autoclaving at 121°C, 15 psi for 15 - 20 minutes.

2

Inoculate Aseptically

Transfer sample using sterile technique to prevent environmental contamination.

3

Incubate

Maintain plates at 37°C (body temperature) for 24 - 48 hours to allow colony formation.

4

Observe & Document

Carefully record colony characteristics including size, color, shape, elevation, and any hemolysis patterns.

5

Compare & Identify

Reference findings against bacterial atlases and known standards for provisional identification.

Colony Characteristics & Clinical Correlation

Each observable colony feature provides diagnostic clues and clinical relevance. Understanding these characteristics enables rapid preliminary identification.

Characteristic	Appearance	Oral Example	Clinical Relevance
Form	Circular / irregular	*Streptococcus mutans*	Dental caries
Elevation	Flat / convex / raised	*Lactobacillus* spp.	Deep caries
Edge	Smooth / rough / wavy	*Actinomyces israelii*	Chronic abscess
Color	White / gray / golden	*S. aureus*	Mucosal infections
Texture	Dry / mucoid / smooth	*Neisseria* spp.	Normal flora
Odor	Foul / fruity / putrid	*Pseudomonas aeruginosa*	Halitosis
Hemolysis	α / β / γ patterns	*S. pyogenes, S. viridans*	Gingival infections

Culture Media Selection & Purpose

Different media support growth of specific bacterial groups. Selecting the correct medium is critical for optimal isolation and presumptive identification.

Blood Agar

Detects hemolysis patterns; supports most oral pathogens including streptococci and staphylococci.

Chocolate Agar

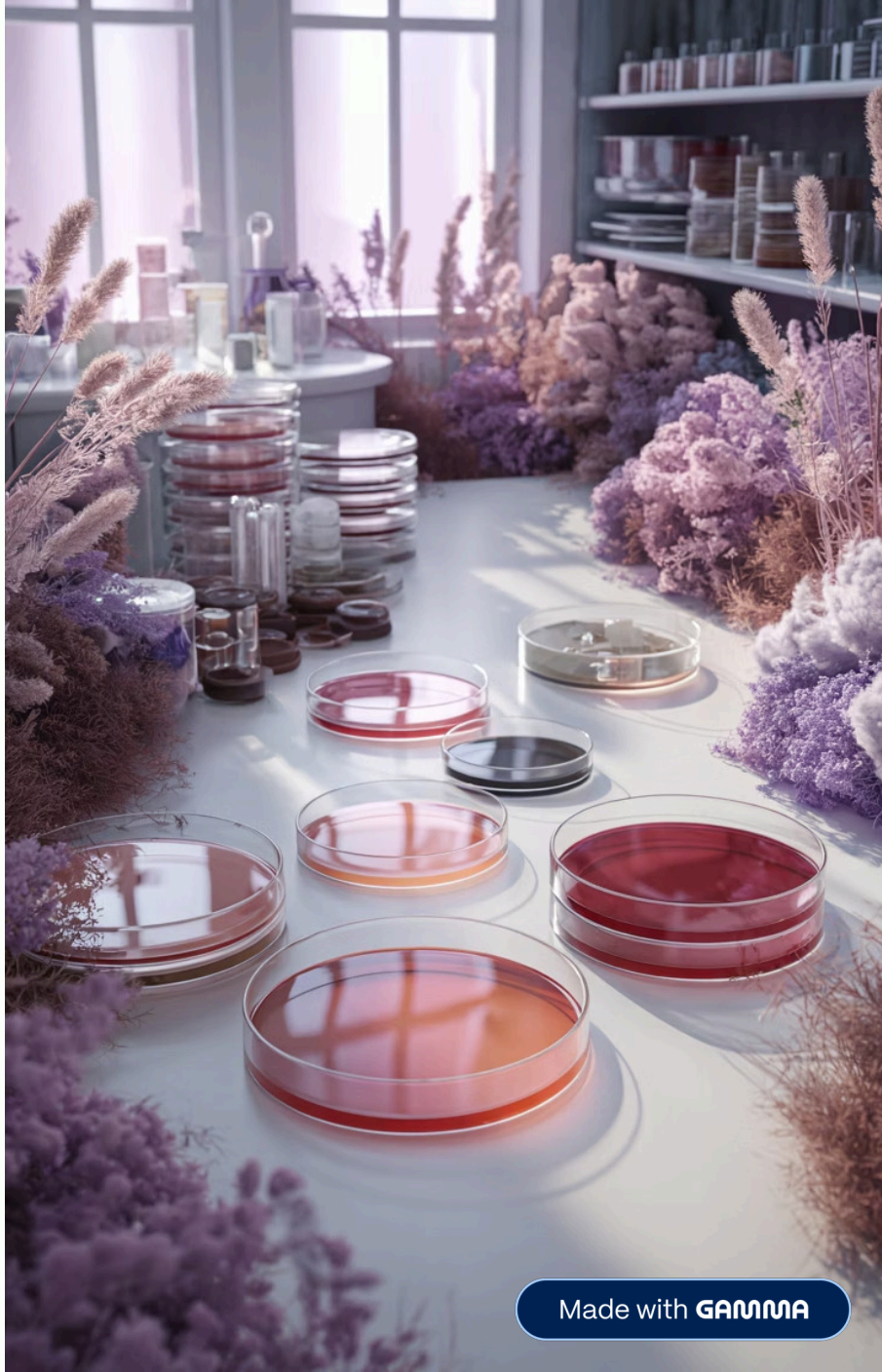
Supports fastidious organisms like *Neisseria* and *Haemophilus* species.

Nutrient Agar

General-purpose medium for non-fastidious bacteria; enables routine culturing.

Sabouraud Agar

Selective for fungi and yeasts like *Candida albicans*; inhibits bacterial growth.



Module 2: Microscopic Examination & Gram Staining

Definition & Purpose

Microscopic examination reveals bacterial morphology, cell arrangement, and staining characteristics. Gram staining is the gold standard, categorizing bacteria as Gram-positive or Gram-negative based on cell wall composition.

Why Gram Staining Matters

This fundamental test guides antibiotic selection, as Gram-positive and Gram-negative bacteria respond differently to specific antibiotics due to cell wall structural differences.

The Gram Staining Procedure

A precise five-step process that differentially stains bacteria based on peptidoglycan layer thickness.

Prepare & Fix

Clean slide and heat-fix sample to adhere bacteria and preserve morphology.

Crystal Violet (1 min)

Primary stain colors all bacteria purple.

Iodine Mordant

Forms crystal violet-iodine complex, trapping dye in thick peptidoglycan.

Alcohol Decolorization

Thin-walled Gram-negative bacteria lose purple color; thick-walled Gram-positive retain it.

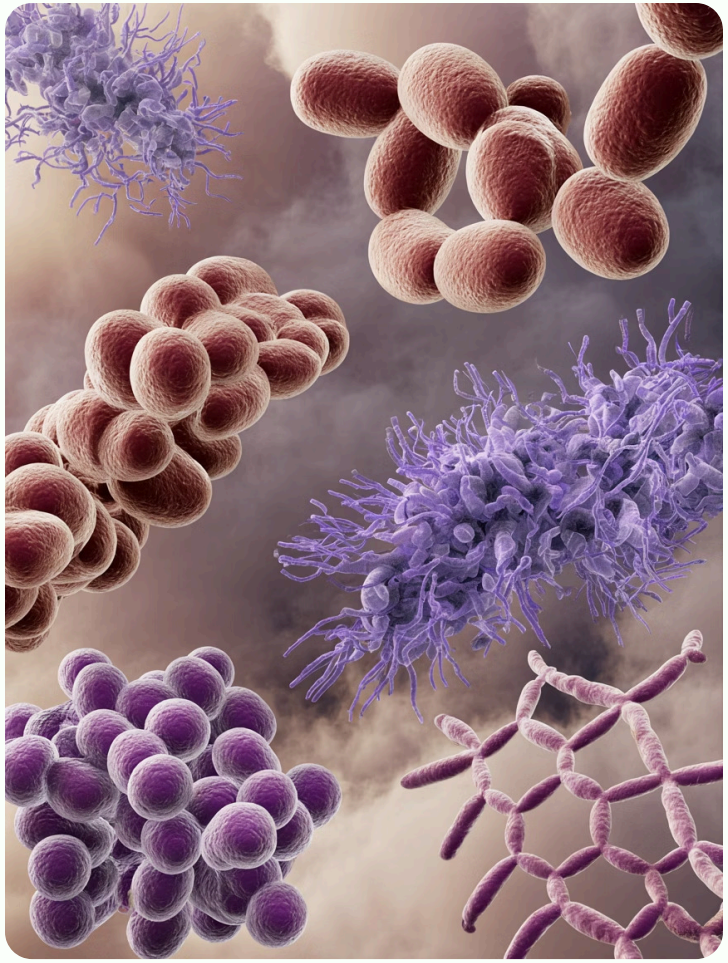
Safranin Counterstain (30 sec)

Red dye colors decolorized Gram-negative bacteria; Gram-positive remain purple-violet. View at 100× oil immersion.

Bacterial Morphology & Clinical Significance

Bacterial shape and arrangement provide critical identification clues and correlate with specific oral diseases and pathogenic mechanisms.

Morphology	Arrangement	Oral Example	Gram Stain	Clinical Significance
Cocci	Singles, pairs, chains	*Streptococcus mutans*	Gram-positive	Initiates dental caries; acid production
Bacilli	Single or paired rods	*Lactobacillus* spp.	Gram-positive	Deep caries progression; acidogenic
Spirochetes	Thin, motile spirals	*Treponema denticola*	Gram-negative	Severe periodontitis; chronic gingivitis
Filamentous	Branched filaments	*Actinomyces israelii*	Gram-positive	Chronic abscess; sulfur granules



Cell Arrangement Patterns

1. *Strepto- (chains): Streptococcus spp.*
2. *Staphylo- (clusters): Staphylococcus spp.*
3. *Diplo- (pairs): Neisseria spp.*
4. *Filamentous branching: Actinomyces spp.*

🔷 Clinical Correlation

Bacteria	Shape	Gram	Oral Location	Related Disease
S. mutans	Cocci	+	Plaque	Dental caries
Lactobacillus spp.	Bacilli	+	Deep caries	Caries progression
Treponema denticola	Spirochete	-	Periodontal pockets	Chronic gingivitis
Actinomyces israelii	Filamentous	+	Tooth root	Chronic abscess
Neisseria spp.	Diplococci	-	Pharynx	Normal oral flora

Final Summary

Type of Examination	Purpose	Result	Clinical Use
Macroscopic	Describe colony	Shape, color, odor	Preliminary diagnosis
Microscopic	Describe shape and Gram	Gram + or -	Confirm diagnosis
Biochemical	Additional tests	Positive / Negative	Precise identification